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ORIGINAL REPORT

Associations of Insulin Resistance and Adiponectin With Mortality in Women With Breast Cancer

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ABSTRACT

Purpose
Overweight or obese breast cancer patients have a worse prognosis compared with normal-weight patients. This may be attributed to hyperinsulinemia and dysregulation of adipokine levels associated with overweight and obesity. Here, we evaluate whether low levels of adiponectin and a greater level of insulin resistance are associated with breast cancer mortality and all-cause mortality.

Patients and Methods
We measured glucose, insulin, and adiponectin levels in fasting serum samples from 527 women enrolled in the Health, Eating, Activity, and Lifestyle (HEAL) Study, a multiethnic, prospective cohort study of women diagnosed with stage I-IIIA breast cancer. We evaluated the association between adiponectin and insulin and glucose levels (expressed as the Homeostatic Model Assessment [HOMA] score) represented as continuous measures and median split categories, along with breast cancer mortality and all-cause mortality, using Cox proportional hazards models.

Results
Increasing HOMA scores were associated with reduced breast cancer survival (hazard ratio [HR], 1.12; 95% CI, 1.05 to 1.20) and reduced all-cause survival (HR, 1.09; 95% CI, 1.02 to 1.15) after adjustment for possible confounders. Higher levels of adiponectin (above the median: 15.5 μg/mL) were associated with longer breast cancer survival (HR, 0.39; 95% CI, 0.15 to 0.95) after adjustment for covariates. A continuous measure of adiponectin was not associated with either breast cancer–specific or all-cause mortality.

Conclusion
Elevated HOMA scores and low levels of adiponectin, both associated with obesity, were associated with increased breast cancer mortality. To the best of our knowledge, this is the first demonstration of the association between low levels of adiponectin and increased breast cancer mortality in breast cancer survivors.

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INTRODUCTION

Overweight and obesity are associated with an increased risk of developing breast cancer and of recurrence or death in breast cancer patients.[1-7] Obesity is commonly associated with hyperinsulinemia (as measured by the Homeostatic Model Assessment [HOMA] score),[8] insulin resistance, and alterations in levels of adipokines such as adiponectin, all of which are associated with an increased risk of developing pre- and postmenopausal breast cancer.[9-12] Some studies have also demonstrated that elevated insulin levels and hyperinsulinemia are associated with poor prognosis in patients with breast cancer.[13,14] Adiponectin is a peptide hormone with levels inversely correlated to body mass index (BMI).[15] Low serum levels of adiponectin are associated with increased risk of breast cancer in both pre- and postmenopausal women and are associated with an aggressive phenotype.[16-19] However, the association of serum adiponectin with breast cancer prognosis is unknown. Here, we examined the association between breast cancer–specific and all-cause mortality and serum levels of insulin, adiponectin, and HOMA score (a measure of insulin resistance) in the Health, Eating, Activity, and Lifestyle (HEAL) Study, a cohort of breast cancer survivors diagnosed with stage I-IIIa breast cancer.

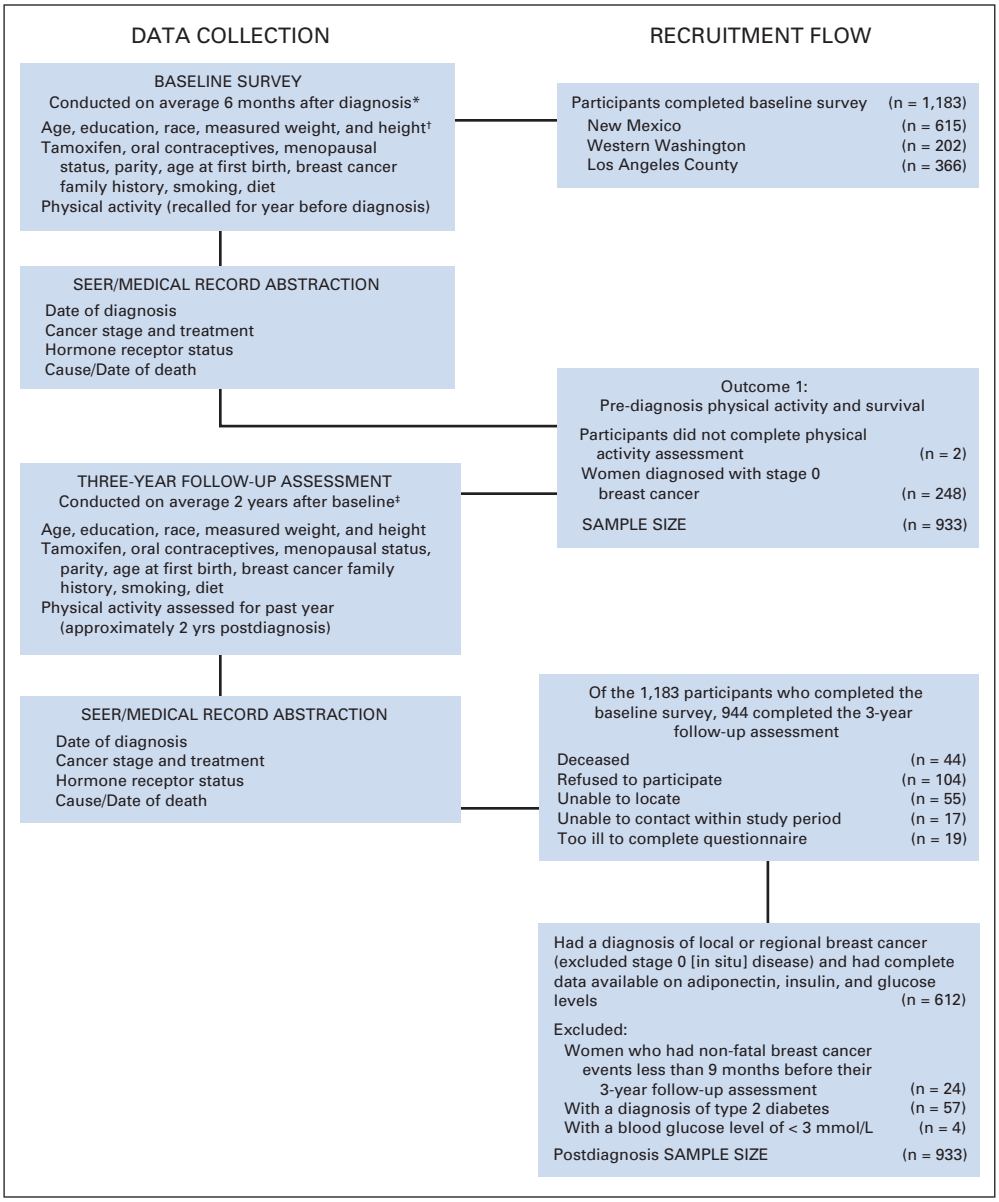

Fig 1. Participant recruitment and timing of data collection. UNM, University of New Mexico; FHCRC, Fred Hutchinson Cancer Research Center; USC, University of Southern California. (*) Survey completed following diagnosis: UNM (mean, 5 months; range, 1 to 9 months), FHCRC (mean, 8 months; range, 3 to 23 months), and USC (mean, 6 months; range, 2 to 17 months). (†) For USC, weight and height 5 years prior to diagnosis were self-reported on baseline survey, ([doubledagger]) Assessment completed post baseline: UNM (mean, 23 months; range 17 to 32 months), FHCRC (mean, 24 month; range, 12 to 29 months), and USC (mean, 27 months; range, 23 to 45 months).

PATIENTS AND METHODS

Study Setting, Participants, and Recruitment

The HEAL Study is a population-based, multicenter, multiethnic prospective cohort study that enrolled 1,183 women diagnosed with breast cancer to evaluate whether diet, weight, physical activity, lifestyle, hormones, or other exposures affect breast cancer prognosis. The aims, study design (Fig 1), and recruitment procedures have been published previously,[20] and we have provided additional information in the Appendix (online only).

The study was performed with the approval of the institutional review boards of participating centers, in accordance with an assurance filed with and approved by the US Department of Health and Human Services. Written informed consent was obtained from each patient. A total of 944 women completed in-person interviews at approximately 3 years post-diagnosis. Of these, 612 had a diagnosis of local or regional breast cancer, with complete data available on adiponectin, insulin, and glucose levels.

An additional 85 women were excluded: 24 had nonfatal breast cancer events < 9 months before their 24-month interview dates and were excluded to avoid potential confounding from possible recent treatment; 57 had a diagnosis of type 2 diabetes; and four had blood glucose levels < 3.0 mmol/L (5.4 mg/dL).[21,22] The final sample was 527 participants.

Data Collection

A 30-mL fasting blood sample was collected from patients at the 24-month interview, processed within 3 hours of collection, and stored at −80°C until analysis. Adiponectin (radioimmunoassay, Linco Research, St. Charles, MI), insulin (Beckman Coulter Unicel DxI Access Ultrasensitive Insulin assay, Beckman, Fullerton, CA), and glucose (Beckman Synchron DxC system, Beckman) were measured. All samples were analyzed within a 2-month time period. Inter-assay coefficients of variation from blind duplicate samples were 18.9%, 3.3%, and 11.3%, and intra-assay coefficients of variation were 16.7%, 2.8%, and 7.5% for adiponectin, insulin, and glucose, respectively.

Covariates

Standardized questionnaire information, including medical history and demographic and lifestyle information, was collected at baseline (corresponding to 6 to 8 months post-diagnosis) and, on average, 30 months post-diagnosis. Information on disease stage, estrogen receptor (ER) status, and

adjuvant therapy was abstracted from medical records. With participants wearing light indoor clothing, weight was measured at approximately 30 months post-diagnosis to the nearest 0.1 kg, and height was measured, without shoes, to the nearest 0.1 cm. All measurements were performed twice and averaged. BMI was estimated as kg/m^2. A race/ethnicity/study site four-category variable was created to adjust for race- and site-associated confounding because these were highly correlated. The variable had four categories: non-Hispanic whites at the University of New Mexico, non-Hispanic whites at the Fred Hutchinson Cancer Research Center, Hispanics, and African Americans. HOMA was first described in 1985[8] and is a method for assessing ß-cell function and insulin resistance and is calculated as (insulin μU/mL $\times$ glucose mmol/L)/22.5.

Stage of Disease and Cancer Treatment

We obtained data on disease stage from the local Surveillance, Epidemiology, and End Results (SEER) registries before recruiting women into the HEAL Study. Participants were classified as having stage 0 (in situ), stage I (localized), or stage II-IIIA (regional) breast cancer, based on the American Joint Committee on Cancer stage of disease classification contained within SEER. (This analysis included only women with stage I-IIIa at diagnosis.) ER status of tumors was categorized as positive, negative, or unknown/borderline. Treatment and additional clinical data were obtained from medical records review. Adjuvant treatment was categorized into four groups: surgery only, surgery and radiation, surgery and chemotherapy, or surgery, radiation, and chemotherapy.

Outcome Assessment

Information on vital status was obtained from SEER. Cause of death codes were acquired from linkages with the relevant SEER database, which obtains data from state and national death certificate files and the Social Security Death Index. If alive, individuals were followed through their last follow-up assessment or SEER vital status update, whichever was most recent. All-cause mortality was defined as time from the 24-month follow-up interview (when serum samples were collected) to death from any cause, or end of follow-up (December, 31, 2006). Breast cancer mortality was defined as death from breast cancer or end of follow-up, with the same intervals as for all-cause mortality.

Statistical Analysis

Correlations between continuous variables were estimated using the Pearson correlation. Differences in distribution between racial/ethnic groups for log-transformed continuous variables were estimated using analysis of variance (ANOVA) with multiple comparison testing (Scheffé F-test). Differences in distribution for categoric variables were estimated using the χ^2 test.

Hazard ratios (HRs) for all-cause mortality or breast cancer mortality and 95% CIs were based on the partial likelihood for Cox's proportional

Table 1. Age and Multivariable Adjusted HR and 95% CI for Associations of Fasting Levels of Adiponectin and HOMA Scores

Variable	No. of Events/No. of Patients	Unadjusted			Model 1*			Model 2†		
		HR	95% CI	P‡	HR	95% CI	P‡	HR	95% CI	P‡
Breast cancer mortality										
Adiponectin, μg/mL				.06			.09			.04
0.85-15.50	19/264	1.00	Ref.		1.00	Ref.		1.00	Ref.	
15.50-74.45	9/263	0.47	0.21 to 1.04		0.47	0.19 to 1.12		0.39	0.15 to 0.95	
Adiponectin, mg/mL				N/S			N/S			N/S
0.85-74.45	28/527	1.00	0.99 to 1.00		1.00	0.99 to 1.00		1.00	0.99 to 1.00	
ln(Adiponectin)	28/527	0.72	0.43 to 1.20	N/S	0.89	0.52 to 1.53	N/S	0.80	0.45 to 1.41	N/S
HOMA				N/S			N/S			.07
0.26-1.03	3/132	1.00	Ref.		1.00	Ref.		1.00	Ref.	
1.04-1.63	8/132	2.67	0.71 to 10.07		2.74	0.69 to 10.80		3.42	0.87 to 13.38	
1.64-2.71	8/132	2.71	0.71 to 10.20		3.69	0.89 to 15.12		4.47	1.07 to 18.57	
2.72-40.16	9/131	3.06	0.83 to 11.29		2.63	0.55 to 12.43		4.27	0.85 to 21.21	
HOMA				.002			.01			.001
0.26-40.16	28/527	1.08	1.03 to 1.13		1.08	1.02 to 1.15		1.12	1.05 to 1.20	
ln(HOMA)	28/527	1.63	1.07 to 2.49	.02	1.58	0.93 to 2.69	.09	1.87	1.07 to 3.29	.03
All-cause mortality										
Adiponectin, μg/mL				N/S			N/S			N/S
0.85-15.50	33/264	1.00	Ref.		1.00	Ref.		1.00	Ref.	
15.50-74.45	29/263	0.88	0.54 to 1.45		0.67	0.38 to 1.17		0.63	0.36 to 1.12	
Adiponectin, μg/mL				N/S			N/S			N/S
0.85-74.45	62/527	1.00	0.99 to 1.00		1.00	0.99 to 1.00		1.00	0.99 to 1.00	
ln(Adiponectin)	28/527	1.10	0.77 to 1.68	N/S	0.93	0.61 to 1.42	N/S	0.90	0.58 to 1.40	N/S
HOMA				N/S			N/S			N/S
0.26-1.03	15/132	1.00	Ref.		1.00	Ref.		1.00	Ref.	
1.04-1.63	15/132	1.03	0.50 to 2.11		0.88	0.41 to 1.85		0.98	0.46 to 2.02	
1.64-2.71	13/132	0.90	0.43 to 1.90		0.98	0.44 to 2.21		1.10	0.48 to 2.50	
2.72-40.16	19/131	1.33	0.67 to 2.61		1.28	0.52 to 3.13		1.60	0.63 to 4.06	
HOMA				.046			.02			.007
0.26-40.16	62/527	1.04	1.00 to 1.10		1.07	1.02 to 1.13		1.09	1.02 to 1.15	
ln(HOMA)	62/527	1.22	0.89 to 1.66	N/S	1.33	0.87 to 2.02	N/S	1.41	0.91 to 2.18	N/S

Abbreviations: HR, hazard ratio; HOMA, Homeostatic Model Assessment; Ref., reference; N/S, not significant.

*Adjusted for age (continuous), body mass index (categorical $<$ 18.5; $\geq$ 18.5 and $<$ 25; $\geq$ 25 and $<$ 40; $>$ 40 kg/m^2), ethnicity/site, and tamoxifen use at time of blood draw (yes/no).

†Adjusted for age (continuous), body mass index (categorical $<$ 18.5; $\geq$ 18.5 and $<$ 25; $\geq$ 25 and $<$ 40; $>$ 40 kg/m^2), ethnicity/site, tamoxifen use at time of blood draw (yes/no), estradiol levels (continuous), leptin levels (continuous), and insulin-like growth factor 1 levels (continuous).

‡Wald test for trend.

hazards model.[23] Tests of the proportional hazards assumption were carried out using Schoenfeld residuals, and they held for all covariates tested.

We estimated the Nelson-Aalen cumulative hazard function with Cox-Snell residuals as the time variable to verify model fit. We thus selected the following covariates from available data for inclusion with the variable of interest: age; race/ethnicity; BMI (categoric < 18.5; ≥ 18.5 and < 25; ≥ 25 and < 40; > 40 kg/m^2); tamoxifen use at time of interview (yes/no); and leptin, insulin-like growth factor 1 (IGF-1), and estradiol levels.

We estimated the relationship between levels of adiponectin and HOMA and breast cancer mortality and all-cause mortality using two different models (Table 1), adjusted for (1) age, ethnicity/site, BMI, and tamoxifen use and (2) model 1 covariates, and leptin, IGF-1, and estradiol. *P* values were estimated using the Wald test for trend.

We examined the association of HOMA and adiponectin in different subgroups: SEER summary stage 2 (local) and stage 3 (regional) tumors; ER-negative and ER-positive tumors; BMI ≤ 25 and > 25 (events were too few to investigate the four-level BMI subgroups); and African American participants versus Hispanic/non-Hispanic whites.

To avoid the possibility of an inflated false-positive rate in multiple subgroup analyses, we applied a stricter criterion of significance of $1\text{-}0.95^{1/K}$, for K-independent tests. We thus used 0.006 to assess statistical significance.[24] All *P* values are two-sided. Analyses were performed using STATA 10 (STATA, College Station, TX).

RESULTS

Characteristics of HEAL participants are shown in Table 2. Median follow-up time was 76.9 months, mean age at 30 months post-diagnosis follow-up was 57.3 years, and mean BMI was 27.3 kg/m^2. Sixty-two deaths occurred among the 527 women, of which 28 were due to breast cancer. Adiponectin values were statistically significantly higher in non-Hispanic whites than among African Americans or Hispanics. HOMA scores were statistically significantly higher in African Americans than among Hispanics or non-Hispanic whites.

Adiponectin correlated inversely with HOMA, waist-to-hip ratio, BMI, and leptin; inversely but weakly with estradiol, testosterone, free estradiol, dehydroepiandrosterone, and estrone; and positively with age (Table 3). In contrast, HOMA correlated positively with BMI and leptin and more weakly with waist-to-hip ratio, dehydroepiandrosterone, and testosterone. Unlike adiponectin, HOMA was negatively correlated with IGF-1.

Table 1 shows the associations between breast cancer–specific and all-cause mortality and between median levels of adiponectin and HOMA score. Higher-than-median levels of adiponectin were associated with a statistically significant decreased risk of breast cancer mortality (HR, 0.39; 95% CI, 0.16 to 0.95). There was no association between levels of adiponectin and all-cause mortality. When analyzed as a continuous variable, adiponectin was not associated with either breast cancer mortality or all-cause mortality.

HOMA, analyzed as a continuous variable, was significantly associated with breast cancer mortality (HR, 1.12; 95% CI, 1.05 to 1.20). HOMA was also associated with shorter all-cause mortality (HR, 1.09; 95% CI, 1.02 to 1.15) but not when analyzed as a log-transformed variable. The magnitude of the association between insulin and risk of breast cancer mortality and all-cause mortality was significant and similar to, if smaller than, that of HOMA (data not shown).

A diagnosis of diabetes is dependent on a repeated measure of fasting glucose of > 126 mg/dL. Seventy-six participants in our cohort had blood glucose levels > 126 mg/dL. While these women did not report a diagnosis of diabetes, and this measure was from a single sample of fasting blood, we reran our analyses excluding these participants. High levels of adiponectin were associated with improved breast cancer survival and all-cause survival in individuals with a glucose level < 126 mg/dL, but HOMA in this restricted sample was not associated with either end point (Table 4).

Patients in the low-glucose group (n = 76) had a mean insulin level of 8.49 μU/mL compared with 16.45 μU/mL in the high-glucose group (ANOVA $P < .001$). The association in the high-glucose group remained significant between HOMA and breast cancer mortality (six deaths; HR, 1.44; 95% CI, 1.11 to 1.88; $P = .006$) and overall mortality (eight deaths; HR, 1.29; 95% CI, 1.11 to 1.51).

We next analyzed the same end points for HOMA and adiponectin (using the same median cutoff) in subgroups of the cohort, using a fully adjusted model. Higher HOMA scores were significantly associated with breast cancer mortality in women diagnosed with SEER stage 3 tumors (HR, 1.20; 95% CI, 1.09 to 1.33; $P < .001$) and ER-positive tumors (HR, 1.19; 95% CI, 1.09 to 1.31; $P < .001$) and in African American women (HR, 1.19; 95% CI, 1.08 to 1.32; $P < .001$). HOMA scores were significantly associated with all-cause mortality, again, in women diagnosed with SEER stage 3 tumors (HR, 1.12; 95% CI, 1.04 to 1.21; $P = .002$), in African American women (HR, 1.15; 95% CI, 1.06 to 1.24; $P = .001$), and in women with a BMI > 25 kg/m^2 (HR, 1.11; 95% CI, 1.04 to 1.19; $P = .003$). HOMA scores were not associated with either breast cancer mortality or all-cause mortality in women diagnosed with SEER stage 2 tumors, BMI ≤ 25 kg/m^2, Hispanic/non-Hispanic white patients, or in women diagnosed with ER-negative tumors. Adiponectin was not predictive of either end point in any subgroup (Appendix Table A1, online only).

We had incomplete data on ER status. However, adjustment of the final model by available ER status attenuated the association between breast cancer mortality and low levels of adiponectin (HR, 0.55; 95% CI, 0.22 to 1.39). HOMA remained associated with both breast cancer mortality (HR, 1.13; 95% CI, 1.05 to 1.22) and all-cause mortality (HR, 1.09; 95% CI, 1.02 to 1.15) after adjustment for ER status.

Results for the postmenopausal women, who comprised the majority of the women in the cohort, were similar to those of the complete cohort (data not shown). Because waist-to-hip ratio is associated with poor all-cause survival in some studies and correlated strongly with both adiponectin and HOMA, we adjusted the model further by incorporating waist-to-hip ratio. This did not affect the results for HOMA, but it significantly attenuated the results for adiponectin, which were no longer significantly associated with decreased breast cancer mortality (data not shown).

DISCUSSION

In this study, we observed that adiponectin levels measured from serum collected from breast cancer survivors approximately 30 months post-diagnosis and above the median in our study sample were associated with a 61% decreased risk of breast cancer–associated death. To the best of our knowledge, this is the first demonstration of an association between high levels of adiponectin and improved breast cancer prognosis.

Additionally we found that elevated HOMA scores were significantly associated with both breast cancer mortality and all-cause-mortality in a model adjusted for age, BMI, race/site, tamoxifen use at

Table 2. Baseline Characteristics of the HEAL Cohort

Characteristic	All*		Non-Hispanic White		African American		Hispanic		Multiple Comparison Across Groups†	
	No.	%	No.	%	No.	%	No.	%	Group	*P*
Total No. of patients	527		325	61.7	130	24.5	60	11.5		
Study site										
Seattle	105		92		0		2			
New Mexico	292		233		0		58		—	
Los Angeles	130		0		130		0			
Adiponectin, μg/mL										< .001‡
Mean	17.2		19.5		12.4		15.4		NHW *v* H	.011
Median	15.5		17.9		9.9		15.4		NHW *v* AA	< .001
IQR	0.85-74.5		1.0-74.5		0.85-57.8		2.0-32.6		H *v* AA	.001
HOMA score										< .001‡
Mean	2.55		1.8		4.8		2.1		NHW *v* H	N/S
Median	1.63		1.4		2.9		1.4		NHW *v* AA	< .001
Range	0.25-40.16		0.26-16.0		0.30-40.2		0.0-23.9		H *v* AA	< .001
BMI, kg/m^2										< .001‡
Mean	27.3		26.1		30.1		27.3		NHW *v* H	N/S
SD	6.0		5.3		7.3		4.8			
Median	26.3		24.9		28.9		26.9		NHW *v* AA	< .001
Range	16.2-53.3		16.3-46.1		16.2-53.3		18.93-41.0		H *v* AA	.008
Waist-to-hip ratio										< .001‡
Mean	0.82		0.81		0.84		0.84		NHW *v* H	.02
SD	0.07		0.07		0.07		0.07			
Median	0.82		0.81		0.85		0.84		NHW *v* AA	< .001
Range	0.64-1.04		0.63-1.04		0.67-1.01		0.72-1.04		H *v* AA	N/S
Age, years										.001‡
Mean	57.33		60.2		51.2		55.7		NHW *v* H	.007
SD	10.9		11.0		7.4		11.5			
Median	56.0		59		51.0		53.0		NHW *v* AA	< .001
Range	31.0-89.0		39.0-89.0		38.0-66.0		31.0-83.0		H *v* AA	.02
Menopausal status at 24-month interview										.02§
Premenopausal	88		45		28		14		—	
Postmenopausal	417		275		87		45			
Unknown	22		0		15		1			
ER status										< .001§
Negative	103		42		48		13		—	
Positive	379		258		75		35			
Unknown	45		25		7		12			
SEER summary stage										< .001§
Local	382		253		73		47			
Regional	145		72		57		13		—	
Treatment at diagnosis										.002§
Surgery	126		76		31		18			
Surgery and radiotherapy	194		136		31		21		—	
Any chemotherapy	207		113		68		21			
Tamoxifen use at 24-month interview										.02§
Yes	251		139		74		35		—	
No	273		183		56		25			
Unknown	3		3		0		0			
Smoking status										N/S§
Current	64		35		21		7			
Former	205		139		39		22		—	
Never	255		148		70		31			
Unknown	3		3		0		0			

Abbreviations: HEAL, Health, Eating, Activity, and Lifestyle Study; NHW, non-Hispanic white; AA, African American; H, Hispanic; IQR, interquartile range; HOMA, Homeostatic Model Assessment; N/S, not significant; BMI, body mass index; SD, standard deviation; ER, estrogen receptor; SEER, Surveillance, Epidemiology and End Results.

*Twelve patients were described as "other race" and were excluded from the statistical comparisons, leaving a total of 515 patients.

†Pair-wise differences for race groups based on the Scheffé test.

‡Overall significant differences among race groups according to analysis of variance (ANOVA).

§χ^2 test.

Table 3. Pearson Correlation (n = 527)

Variable	r	
	Adiponectin	HOMA Score
Adiponectin	—	−0.38*
HOMA score	−0.38*	—
BMI at 24-month interview	−0.28*	0.52*
Waist-to-hip ratio (n = 510)	−0.24*	0.38*
Age at 24-month interview	0.23*	−0.05
IGF-1	0.06	−0.20*
IGFBP-3	−0.03	0.01
Leptin	−0.24*	0.50*
Estradiol (n = 522)	−0.11†	−0.03
Free estradiol (n = 522)	−0.13†	0.01
DHEA	−0.17*	0.11*
Estrone	−0.18*	0.04
Testosterone (n = 526)	−0.18*	0.19*

NOTE. All anthropomorphic data measured at the same time point that the blood sample was taken.

Abbreviations: HOMA, Homeostatic Model Assessment; BMI, body mass index; IGF-1, insulin-like growth factor 1; IGFBP-3, insulin-like growth factor binding protein 3; DHEA, dehydroepiandrosterone.

*$P < .001$.

†$P < .01$.

time of blood draw, and levels of leptin, estradiol, and IGF-1. However, unlike Goodwin et al,[13] who reported an HR of 3.3 for risk of death in patients with insulin levels in the highest quartile compared with the lowest, we found no association with outcome when we analyzed HOMA scores categorized as quartiles for all-cause mortality. Our means for insulin were higher and had a wider range than those reported by Goodwin et al[25] (66.9 pmol/mL; range, 8.3 to 676.4 pmol/L *v* 8.0 to 340 pmol/L), and the HEAL participants had a higher mean BMI (27.3 kg/m^2 *v* 25.5 kg/m^2).[25] Given the small number of events, we postulate that we have insufficient power to analyze the association with overall mortality by quartiles. For breast cancer mortality, we observed that patients with HOMA levels in the third quartile had a significantly elevated risk of death compared with patients in the lowest quartile. However, the CIs were wide because of the small number of events.

While HOMA scores were associated with all-cause mortality, adiponectin levels were not. However, when we restricted the analysis to participants with glucose levels < 126 mg/dL, high levels of adiponectin were statistically significantly associated with improved prognosis for all-cause mortality (HR, 0.51; 95% CI, 0.28 to 0.91). The lack of association between HOMA scores and outcome in women with glucose levels ≤ 126 mg/dL may be due to lower levels of insulin in this group compared with those in the high-glucose group who are more likely to have a worse prognosis associated with elevated insulin levels. A recent paper examining severe insulin resistance and hyperinsulinemia in a non-obese diabetic mouse model demonstrated accelerated mammary gland development and breast cancer progression independent of obesity and inflammation, mediated via insulin, insulin receptor/IGF-1 receptor, and the PI3K/Akt pathway,[26] supporting the tumor-promoting effect of elevated circulating insulin levels.

As we hypothesized, there was an association between HOMA scores and all-cause mortality in women with a BMI > 25 kg/m^2. Additionally, HOMA was associated with all-cause mortality and breast cancer mortality in participants diagnosed with regionally invasive and ER-positive breast cancer. Adiponectin was not associated with outcome in any of these subgroups.

Previous studies[13,14] have demonstrated that hyperinsulinemia is associated with poor prognosis in women diagnosed with breast cancer. Insulin can stimulate cell proliferation, possibly via signaling through its receptor,[27] in both normal and malignant breast cell lines.[28] In addition, insulin downregulates IGF binding proteins and sex hormone–binding globulin, thus increasing bioavailable mitogens such as sex steroid hormones and IGF-1 with accompanying downstream cellular effects.[29-32] Our observation of a greater association of mortality with HOMA, a marker of insulin resistance and long-term hyperinsulinemia, compared with insulin concentrations, may be because a single measure of insulin is an incomplete marker of long-term insulin production and may not reflect diurnal variation. In support of this, an earlier report from our study found that elevated C-peptide concentrations were negatively associated with breast cancer survival (Irwin et al, submitted for publication).

Epidemiologic studies support a significant inverse association between adiponectin and breast cancer that is present even with adjustment for adiposity.[16-19,34,35] Adiponectin is secreted from adipose tissue and may play a key role in regulating energy intake and expenditure.[36] Levels are inversely correlated with BMI and adipose tissue mass[15,37,38] and are downregulated in overweight/obesity.[37,39-43]

Comparable to results from another study,[44] our results demonstrated that adiponectin is inversely associated with sex steroid levels, which have positive associations with progression of breast cancer. Adiponectin has effects on cell proliferation and cytokine production,[45] and experiments both in vitro and in animal models suggest an interaction between adiponectin and ERs.[46-49] Adiponectin is secreted primarily by adipocytes, which may explain the attenuation of the relationship between adiponectin and breast cancer mortality, after adjustment for central obesity (waist-to-hip ratio).

Our study has several strengths. It is a population-based, multiethnic cohort of women with incident breast cancer; thus, results can be generalized to patients seen in routine clinical practice. Blood was collected after primary treatment was completed and therefore reflects ongoing host factors that can affect prognosis. We were able to adjust results for multiple demographic, anthropometric, tumor, and treatment characteristics, which improved validity.

Our study also has several limitations. We collected only one fasting blood sample and therefore cannot completely characterize the women's exposure to insulin or adiponectin. However, other studies indicate that a single fasting blood measure of these analytes is highly reproducible in postmenopausal women. We could not assess the effect of change in insulin resistance or adiponectin, which would require testing interventions to change these analytes such as weight loss, physical activity, or medications to alter insulin resistance. Finally, we were unable to assess risk in specific subgroups, including ER-negative disease, because of small numbers.

Insulin levels can be successfully lowered via behavioral and lifestyle interventions, such as increasing physical activity levels. In a cohort of breast cancer survivors, a mixed strength and endurance exercise intervention in obese, sedentary breast cancer survivors resulted in significant reductions in circulating insulin levels, and a concomitant nonsignificant improvement in HOMA scores.[50] It is unclear whether alterations in adiponectin levels could be achieved by similar lifestyle interventions.[51] A recent study of overweight breast

Table 4. Analysis Restricted to Women With Blood Glucose Level < 126 mg/dL (n = 451)

Variable	No. of Events/ No. of Patients	Unadjusted			Model 1*			Model 2†		
		HR	95% CI	P‡	HR	95% CI	P‡	HR	95% CI	P‡
Breast cancer mortality										
Adiponectin, μg/mL				.02			.01			.003
0.85-15.50	16/212	1.00	Ref		1.00	Ref		1.00	Ref	
15.50-74.45	6/239	0.33	0.13 to 0.83		0.26	0.10 to 0.73		0.21	0.07 to 0.46	
Adiponectin, μg/mL				N/S			N/S			N/S
0.85-74.45	22/451	0.99	0.99 to 1.00		0.99	0.99 to 1.00		0.99	0.99 to 1.00	
HOMA				N/S			N/S			N/S
0.26-1.22	7/172	1.00	Ref.		1.00	Ref.		1.00	Ref.	
1.23-2.26	10/163	1.51	0.57 to 3.97		1.89	0.68 to 5.26		2.40	0.84 to 6.83	
2.27-23.91	5/116	1.06	0.33 to 3.35		1.32	0.23 to 5.71		1.12	0.31 to 6.42	
HOMA				N/S			N/S			N/S
0.25-23.91	22/451	1.05	0.89 to 1.23		1.04	0.86 to 1.24		1.05	0.87 to 1.27	
All-cause mortality										
Adiponectin, μg/mL				N/S			.046			.02
0.85-15.50	29/212	1.00	Ref		1.00	Ref.		1.00	Ref.	
15.50-74.45	25/239	0.75	0.44 to 1.29		0.56	0.31 to 0.99		0.51	0.28 to 0.91	
Adiponectin, μg/mL				N/S			N/S			N/S
0.85-74.45	54/451	1.00	0.99 to 1.01		1.00	0.99 to 1.01		1.00	0.99 to 1.01	
HOMA				N/S			N/S			N/S
0.26-1.22	21/172	1.00	Ref.		1.00	Ref.		1.00	Ref.	
1.23-2.26	19/163	0.98	0.53 to 1.83		0.92	0.48 to 1.79		1.01	0.51 to 1.96	
2.27-23.91	14/116	1.01	0.52 to 1.99		0.88	0.37 to 2.06		0.96	0.40 to 2.30	
HOMA				N/S			N/S			N/S
0.25-23.91	54/451	1.04	0.92 to 1.16		1.04	0.91 to 1.18		1.06	0.92 to 1.21	

NOTE. As described in Methods, we used a stricter criterion to assess statistical significance in subgroup analyses ($P < .006$).
Abbreviations: HR, hazard ratio; Ref., reference; N/S, not significant; HOMA, Homeostatic Model Assessment.
*Adjusted for age (continuous), body mass index (categorical < 18.5, ≥ 18.5 and < 25, ≥ 25 and < 40, > 40 kg/m^2), ethnicity/site, and tamoxifen use at time of blood draw (yes/no).
†Adjusted for age (continuous); body mass index (categorical < 18.5, ≥ 18.5 and < 25, ≥ 25 and < 40, > 40 kg/m^2), ethnicity/site, tamoxifen use at time of blood draw (yes/no), estradiol levels (continuous), leptin levels (continuous), and insulin-like growth factor 1 levels (continuous).
‡Wald test for trend.

cancer survivors failed to demonstrate any significant changes in adiponectin levels after a 16-week randomized controlled exercise intervention. However, there were also no significant changes in either weight or body composition in this study.[52] Metformin acts through the AMPK/mTOR/S6K1 axis,[53] and observational cohort studies suggest that metformin is associated with reduced risk of cancer in patients with type 2 diabetes.[54-56] Diabetic breast cancer patients using metformin, an insulin-lowering medication, experience a higher rate of pathologic complete response to neoadjuvant chemotherapy than those using other diabetic treatments (odds ratio, 2.95; $P = .04$).[57] This has prompted clinical trials of metformin, including a trial of neoadjuvant chemotherapy and trastuzumab with or without metformin in women diagnosed with HER2-positive primary breast cancer.[58] Two other trials are planned: one that will evaluate the effects of metformin on breast cancer outcomes[59] and a second presurgical, randomized, phase II biomarker trial to evaluate the activity of metformin on tumor cell proliferation in breast cancer patients.[60]

Our study confirms the importance of hyperinsulinemia as an independent risk factor for poor prognosis in women with breast cancer and the association between low levels of adiponectin and shorter breast cancer survival. Randomized controlled trials involving lifestyle interventions, such as physical activity or weight loss, have resulted in reductions in insulin among women with breast cancer and may represent an important approach for improving prognosis in women via reductions in hyperinsulinemia.

AUTHORS' DISCLOSURES OF POTENTIAL CONFLICTS OF INTEREST

The author(s) indicated no potential conflicts of interest.

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